THE RELATION BETWEEN TEMPERATURE, SALTS, HYDROGEN ION CONCENTRATION AND FREQUENCY OF INGESTION OF FOOD BY AMOEBA

A DISSERTATION SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

By

RICHARD A. FENNELL

June, 1936

Reprinted for private circulation from
Physiological Zoölogy, Vol. XI, No. 1, January, 1938

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Physiological Zoölogy

Vol. XI JANUARY 1938 *No. 1*

THE RELATION BETWEEN TEMPERATURE, SALTS, HYDROGEN ION CONCENTRATION, AND FREQUENCY OF INGESTION OF FOOD BY AMOEBA[1]

(Eight figures)

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INTRODUCTION

IT IS well known that under some conditions amoebae do not feed, although the culture medium contains an abundance of food, and that under other conditions some feed and others do not. This shows that feeding in amoebae depends upon the condition of the organisms and the condition of the environment. However, only a few detailed observations have been made concerning the determining factors.

Schaeffer (1916) maintains that the most important single factor in inducing feeding in amoebae is movement of the food, but that the character of the food particles taken in is also involved. He says that, in general, soluble particles are more readily taken than insoluble ones.

Mast and Hahnert (1935) came to the following conclusion:

> *Amoeba proteus* does not feed when it is not attached to the substratum, therefore anything which facilitates attachment facilitates feeding. It has been repeatedly observed that feeding increased greatly if amoebae were transferred from a solution in which attachment was weak, to one in which it was strong, e.g., fresh Chalkley solution. It was repeatedly observed that feeding increased if the amoebae were agitated. This was particularly marked in specimens transferred from ordinary culture dishes to slides. Starvation facilitates feeding, but not if it continues until the amoebae no longer attach firmly. Specimens kept without food 5 to 8 days usually do not feed again no matter how they are treated, although they live 10 days or longer.

Aside from these facts, little is known concerning the factors which determine feeding in *Amoeba*. More is known, however, concerning these factors in other protozoa.

Schaeffer (1910) made a very thorough study of feeding in *Stentor caeruleus*. He maintains that hunger accelerates frequency of ingestion, that satiety retards it, and that *Stentor* takes some organisms more readily than others.

[1] We are indebted to the Marine Biological Laboratory at Woods Hole, Massachusetts, for facilities used in making some of the experiments considered below.

Metalnikow (1912) maintains that low temperatures, high temperatures, weak alkaline solutions, and old culture solutions retard frequency of ingestion of particles by paramecia, and that moderate temperatures, weak acid solutions, alcohols, and arsenious compounds accelerate it. He found also that paramecia ingests digestible substances more readily than indigestible substances.

MATERIALS AND METHODS

Kaulbaum's "purest" chemicals and triple (Pyrex glass) distilled water were used in all solutions. The salts were carefully dried and weighed, and in some instances purified by recrystallization. The concentration of the hydroxide solutions used was ascertained by titrating against a known amount of potassium acid phthalate ($KHC_8H_4O_4$), as outlined by Clark (1928). The solutions were stored in Pyrex glass flasks, which contained an outlet with a stopcock and an inlet with a soda-lime tube attached. The concentration of $MgCl_2$ and $CaCl_2$ was ascertained by the method described by Blasdale (1931). The concentration of the phosphates used was ascertained by the molybdenum-blue method of Deniges, as modified by Farber and Youngburg (1932). Calcium chloride was substituted for $CaH_4(PO_4)_2$ in solutions with low hydrogen ion concentrations, so as to avoid precipitation of the calcium.

The temperature of the solutions in which feeding was tested was controlled by means of a temperature bath, designed by Pitts (1932). This bath consists of a small brass box which fits on the stage of the microscope and contains a flat-bottom glass dish surrounded by a rubber diaphragm to prevent leakage of water. The glass dish was made as follows: A 50-cc. Pyrex glass beaker was painted inside and outside with paraffin; then a line in the paraffin 2 mm. wide was made around the beaker 2.5 cm. from the bottom, after which it was immersed in hydrofluoric acid and left 24 hours. During this time the acid dissolved the glass which was not covered by paraffin, separating the bottom from the top. The rough edges of the bottom were ground down and polished. The temperature in the glass dish was adjusted by regulating the flow of water of the desired temperature through the bath. The hydrogen ion concentration of the solutions was measured electrically.

Chilomonas paramecium cultured in sterile glucose-peptone solution (1.5 g. glucose and 3.5 g. peptone in 1,000 cc. water) was used as food in all experiments made. Sterile cultures were obtained by a method similar to that which was perfected by Hargitt and Fray (1917) and Phillips (1922). Twenty-five chilomonads were put into 0.4 cc. of glucose-peptone solution on a sterile depression slide in a sterile Petri dish. Then the dish was covered and left 5 minutes. The chilomonads were then transferred with a sterile pipet (plugged with sterile cotton at the bulb end) to the same amount of solution on another depression slide in another Petri dish, which was covered and left 5 minutes. This process was then repeated thirteen times, after which one of the chilomonads was transferred to each of fourteen sterile 125 cc. flasks, each of which contained 50 cc. of sterile glucose-peptone solution.

The flasks were left from 5 to 7 days; then one of the sterile flasks was used to inoculate additional flasks, each containing 50 cc. of sterile glucose-peptone solution. The freshly inoculated flasks were left 48 hours, that is, until chilomonads were abundant. Then the chilomonads in three of these flasks were transferred, as follows, to the solution used in the tests on feeding: Fifteen cubic centimeters of the solution in one of the flasks was put into each of two centrifuge tubes and centrifuged at a moderate speed for

5 minutes, after which all but 1 cc. of the solution (i.e., the solution which contained the chilomonads) was poured off. Then 14 cc. of the solution from the flask was added to each test tube. This was repeated until all the solution in the three flasks was centrifuged. Then 14 cc. of the solution to be used for the test on feeding was added to each tube and centrifuged, after which all but 1 cc. at the bottom of the tube was poured off. This procedure was repeated three times. During the successive transfers the glucose-peptone in which chilomonads were cultured was diluted about 40,000 times. Chilomonads were prepared in this way for each test on feeding, as described below.

The amoebae used were cultured in Chalkley solution (see p. 4) containing rice and wheat. Three weeks after inoculation the best culture was selected; and from this 250 amoebae[2] of the same size and appearance were taken, put into a small Pyrex glass dish which contained 5 cc. distilled water, and left 10 minutes. Then they were transferred through the same amount of distilled water three more times, but after the last transfer they were left for 24 hours instead of 10 minutes. At this time each amoeba had three or four long, slender pseudopods and little, if any, food (no fat or starch), and most of them were not attached or only loosely attached to the substratum. From these 250 amoebae, 100 as nearly as possible the same in size, structure, and form were selected and put into a small beaker which contained 5 cc. of the solution to be used in the test on feeding, and left 5 minutes. Then they were transferred, in the same way, through the same amount of solution three more times, after which they were transferred to the same amount of solution in the small glass dish in the Pitts temperature bath, which was now covered, sealed with vaseline, and left undisturbed for 15 minutes for adjustment to the solution. Then the cover of the dish was removed, and 2 cc. of the solution containing chilomonads prepared for tests on feeding were added, after which the dish was again covered and sealed. Fifteen minutes after adding the chilomonads to the bath, 10 amoebae were taken out, put on a glass slide between parallel ridges of vaseline, and covered with a cover slip. Then some of the solution was removed with filter paper so as to flatten the amoebae; then the chilomonads in each amoeba were counted, and the mean calculated and recorded. This was repeated 30, 60, and 120 minutes after the chilomonads had been in the Pitts bath. The results obtained are considered in the following pages.

THE RELATION BETWEEN ABUNDANCE OF FOOD AND FREQUENCY OF INGESTION BY *Amoeba proteus*

Metalnikow (1912) maintains that in paramecia the rate of feeding is, within wide limits, independent of the abundance of food in the culture solution; but Fenn (1921) maintains that in leucocytes the number of particles ingested by them is directly proportional to the number of particles in suspension.

One hundred selected amoebae in Chalkley solution were put into each of two Pitts temperature baths, adjusted to 24° C. Then about 925,000 chilomonads were added to one and 1,850,000 to the other, after which Chalkley solution was added until the volume in both was 3 cc.; then the number of chilomonads ingested by the amoebae was ascertained, as described above.

The results are given in Table 1. These results show that the number of chilomonads

[2] Each time that amoebae were removed from a culture, the solution taken out with them was replaced with distilled water. Owing to this, the hydrogen ion concentration in the cultures remained nearly constant.

ingested in 15- and 30-minute periods was slightly higher in the solution which contains the greater number of chilomonads, but that the number ingested in 60 and 120 minutes was nearly the same in both solutions. This demonstrates that there can be great variation in the abundance of chilomonads in the solution without appreciably affecting the number of chilomonads ingested by the amoebae, if they are left half an hour or longer.

THE RELATION BETWEEN TEMPERATURE AND FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus and Amoeba dubia*

Observations were made on ingestion of chilomonads by *A. proteus* in Chalkley solution,[3] and by *A. dubia* in Hahnert (1932) solution, at temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. These observations were made as described in the preceding pages.

TABLE 1

THE RELATION BETWEEN ABUNDANCE OF FOOD AND FREQUENCY OF INGESTION BY *Amoeba proteus* (6 TO 10 AMOEBAE USED IN EACH TEST)

Number of Chilomonads per Cubic Centimeter	Average Number of Chilomonads Ingested by 6–10 Amoebae				Numbers Ingested per Individual in 120 Min.	
	15 Min.	30 Min.	60 Min.	120 Min.	Lowest Number	Highest Number
600,000	12	41	56	80	63	96
300,000	9	36	57	81	64	98

Three sets of observations were made, two on *A. proteus* and one on *A. dubia*. The results obtained in the first set of observations on *A. proteus* and those obtained in observations on *A. dubia* are presented in Table 2 and Figure 1. Figure 1 shows, in these two sets of observations, that, as the temperature increased, the frequency of ingestion of chilomonads by *A. proteus* and by *A. dubia* increased to a maximum at about 26° and then decreased to zero at 40°, but that the number ingested by *A. proteus* in 120 minutes decreased to a median minimum at 30°, and to a second maximum at 35°, before it decreased to zero. Similar results were obtained in the second set of observations on *A. proteus*, but there was no median minimum. The median minimum obtained in the first set is therefore probably not significant. The results as a whole, show that frequency of ingestion in amoebae is closely correlated with temperature.

Table 2 shows that at 25° an average of 63 chilomonads per amoeba were ingested in 120 minutes and that, of these, 26 were ingested during the first 15 minutes, 10 during the next 15 minutes, 20 during the next 30 minutes, and only 7 during the last 60 minutes. The frequency of ingestion therefore decreased from 1.73 chilomonads per minute during the first 15 minutes, when the amoebae contained from 0 to 25 chilomonads each, to only 0.1 chilomonad per minute during the last 60 minutes, when they contained from 56 to 63 each. Table 2 shows also that similar results were obtained at 5°, 10°, 15°, 20°, 25°, and 30° C. Similar results were also obtained in the second set of

[3] Chalkley solution: 80 mg. NaCl, 4 mg. $NaHCO_3$, 4 mg. KCl, 4 mg. $CaCl_2$, 2 mg. $CaH_4(PO_4)_2$, 2 mg. $Mg_3(PO_4)_2\,4H_2O$, and 1,000 cc. distilled water. Hahnert solution: 4 mg. $NaHCO_3$, 4 mg. KCl, 4 mg $CaCl_2$, 2 mg. $CaH_4(PO_4)_2$, 2 mg. $Mg_3(PO_4)_24H_2O$, and 1,000 cc. distilled water.

tests with *A. proteus* and in the set of tests with *A. dubia*. It is consequently evident that the frequency of ingestion varies inversely with the amount of food in the amoebae.

TABLE 2

THE RELATION BETWEEN TEMPERATURE AND THE FREQUENCY OF INGESTION OF CHILOMONADS BY *Amoeba proteus*

Temperature in Degrees C.	Average Number of Chilomonads Ingested by 10 Amoebae				Numbers Ingested per Individual in 120 Min.	
	15 Min.	30 Min.	60 Min.	120 Min.	Lowest Number	Highest Number
5	0.8	0.6	6	9	4	16
10	1.1	7	12	13	6	25
15	2	10	18	43	32	56
20	7	19	37	50	38	60
25	26	36	56	63	46	72
30	25	43	50	51	42	65
35	13	10	43	68	46	86
40	0	0	0	0	0	0

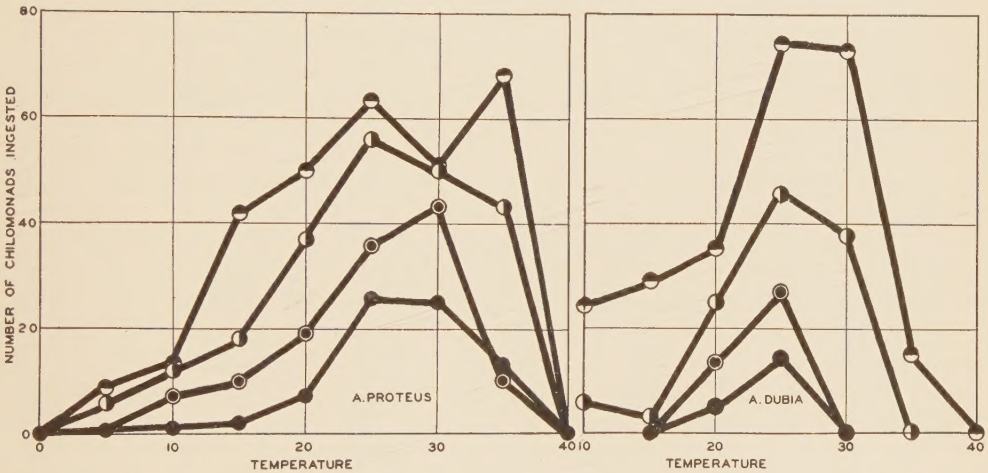

FIG. 1.—The relation between temperaure and frequency of ingestion of chilomonads by *Amoeba*. ●, number of chilomonads ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals. The extent of individual variation in *A. proteus* is presented in Table 2. The variation in *A. dubia* was practically the same.

Table 2 shows that at 35° an average of 13 chilomonads were ingested during the first 15 minutes, none during the next 15 minutes, 33 during the next 30 minutes, and 25 during the last 60 minutes, i.e., that the frequency of ingestion decreased from 0.86 chilomonads per minute during the first 15 minutes to zero during the second, then in-

creased to 1.10 during the next 30 minutes, and then decreased to 0.41 during the last 60 minutes. These results indicate that the frequency of ingestion at 35° is not closely correlated with the number of chilomonads in the amoebae. This is probably due to variations in attachment to the substratum, for the amoebae were fairly firmly attached during the first 15 minutes, when the frequency of ingestion was high, and only loosely during the second 15 minutes, when it was low, but were again fairly firmly attached after that, when it was again high. This indicates that the frequency of ingestion varies directly with the degree of attachment to the substratum, and that the increase in frequency after exposure of 30 minutes at 35° is due to adjustment, resulting in increase in the degree of attachment.

Mast and Prosser (1932) maintain that, as the temperature increases, the rate of locomotion in *A. proteus* increases to a maximum at about 25°, then decreases to a median minimum at about 28°, then increases to a second maximum at about 30°, and then rapidly decreases to zero. Schwitalla (1925) obtained results in accord with these views. This indicates that rate of locomotion is correlated with temperature in much the same way as frequency of ingestion.

THE RELATION BETWEEN HYDROGEN ION CONCENTRATION IN SOLUTIONS CONTANING SODIUM AS THE ONLY METALLIC CATION AND FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus*

The experiments described in this section were made with two series of solutions which consisted of NaH_2PO_4 and NaOH in water. Each of the two series was prepared by mixing NaH_2PO_4 of a given molecular concentration with NaOH of the same molecular concentration in such proportions as to produce a series of hydrogen ion concentrations from pH 4.4 to 8.3. The concentration of the sodium was consequently the same in all the solutions in each of the two series. It was 0.002 N in one and 0.005 N in the other. To obtain hydrogen ion concentrations of pH 4.4, a small amount of 0.1 M HCl was added. Observations were made as described in the preceding pages. The results obtained are presented in Table 3 and Figure 2.

Figure 2 shows that, as the hydrogen ion concentration decreased, the frequency of ingestion of chilomonads by amoebae increased from zero at pH 4.4, nearly to a secondary maximum at pH 5.0, then remained nearly constant to pH 5.5, then increased to a primary maximum between pH 6.0 and 6.5, then decreased to zero at pH 7.7–8.3. It shows that the rate of ingestion in the stronger solution of sodium was consistently much higher than in the weaker solution and that there was somewhat of a median minimum near pH 6.5 in both concentrations.

Figure 2 indicates that frequency of ingestion is more closely correlated with the hydrogen ion concentration in the stronger than in the weaker solution, and that the maximum acidity at which ingestion occurred was the same in both concentrations but that the maximum alkalinity[4] at which it occurred was higher in the weaker than in the stronger solution.

Table 3 columns 6 and 7, show that there was marked individual variation in the frequency of ingestion in the weaker solution but that the averages are consistent throughout. The variation in the stronger solution was essentially the same as in the weaker.

[4] "Maximum alkalinity" is defined as the lowest hydrogen ion concentration in which ingestion occurs; and "maximum acidity," as the highest in which it occurs.

TABLE 3

THE RELATION BETWEEN FREQUENCY OF INGESTION OF CHILOMONADS BY *Amoeba proteus* AND HYDROGEN ION CONCENTRATION IN SOLUTIONS CONTAINING SODIUM AS THE ONLY METALLIC CATION (CONCENTRATION OF SODIUM, 0.002 N)

Hydrogen Ion Concentration in pH Units	Average Number of Chilomonads Ingested by 10 Amoebae				Numbers Ingested per Individual in 120 Minutes	
	15 Min.	30 Min.	60 Min.	120 Min.	Lowest Number	Highest Number
4.4	0	0	0	0	0	0
4.7	16	31	34	43	32	56
5.0	22	34	37	53	39	68
5.5	21	26	36	52	35	74
6.0	23	34	44	51	23	78
6.3	20	29	47	53	35	72
6.6	18	22	33	67	53	76
7.0	19	23	40	47	40	58
7.4	21	38	49	49	20	110
7.8	18	29	42	50	28	70
8.1	17	19	32	44	28	65
8.4	0	0	0	0	0	0

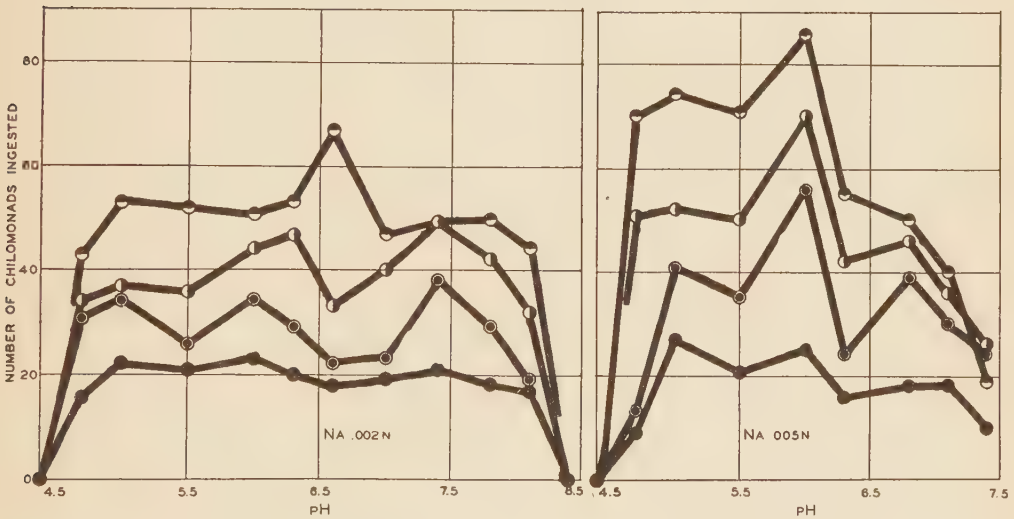

FIG. 2.—The relation between frequency of ingestion of chilomonads by *A. proteus* and hydrogen ion concentration in solutions containing sodium as the only metallic cation. ●, number ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals. Lowest and highest numbers of chilomonads ingested per individual in 120 minutes is 32 and 56, respectively, at pH 4.7; 53 and 76 at pH 6.6, the optimum hydrogen ion concentration. Similar individual variations occurred in all the other tests.

THE RELATION BETWEEN HYDROGEN ION CONCENTRATION IN SOLUTIONS CONTAINING POTASSIUM AS THE ONLY METALLIC CATION AND FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus*

The methods used in making observations on the effect of potassium on frequency of ingestion of food by amoebae were the same as those used in observations on the effect of sodium. The results are presented in Figure 3.

Figure 3 shows that as the hydrogen ion concentration increased the frequency of ingestion of chilomonads increased from zero at pH 4.4 to a maximum at pH 5.0 and then decreased nearly to zero, but that it decreased much more gradually in the weaker than in the stronger solution and that the maximum alkalinity was considerably higher

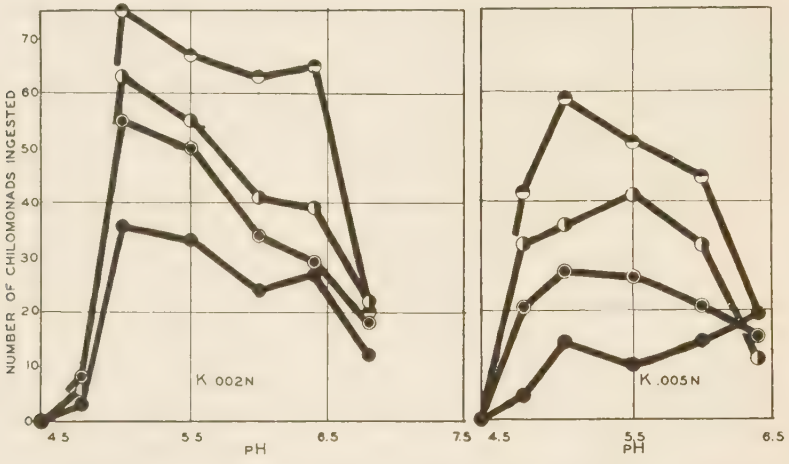

FIG. 3.—The relation between frequency of ingestion of chilomonads by *A. proteus* and hydrogen ion concentration in solutions containing potassium as the only metallic cation. ●, number ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals. The individual variation was similar to that in solutions containing sodium in place of potassium.

in the former than in the latter. It indicates that frequency of ingestion of food is more closely correlated with hydrogen ion concentration in the stronger than in the weaker solutions of potassium, as it is in solutions of sodium.

It will be seen by comparing Figure 3 with Figure 2 that the results obtained with potassium are similar to those obtained with sodium -that in both, the alkaline limit for ingestion is more acid in the stronger than in the weaker solution, but that at the optimum for frequency of ingestion, the solutions which contain potassium are more acid than those which contain sodium, and that, as the concentration increased, frequency of ingestion decreased in the former and increased in the latter.

THE RELATION BETWEEN HYDROGEN ION CONCENTRATION IN SOLUTIONS CONTAINING CALCIUM AS THE ONLY METALLIC CATION AND FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus*

The methods used in making observations on the effect of calcium on frequency of ingestion of food by amoebae were the same as those used in observations on the effect

of sodium, except that $CaCl_2$ was substituted for $CaH_4(PO_4)_2$ in all solutions with hydrogen ion concentrations lower than pH 6.4, because calcium is precipitated in these solutions if phosphates are present, and that the concentration of calcium in the two series of solutions was 0.001 M and 0.0025 M instead of 0.002 M and 0.005 M, and that the observations (owing to increase in hydrogen ion concentration in the alkaline solutions) were continued for only 30 minutes in place of 120 minutes. The results are presented in Figure 4.

Figure 4 shows that, as the hydrogen ion concentration decreased, the frequency of ingestion of chilomonads gradually increased to a maximum approximately at pH 6.0, then remained constant until pH 7.0 was reached, then decreased until the close of the experiment at pH 8.0 but not to zero.

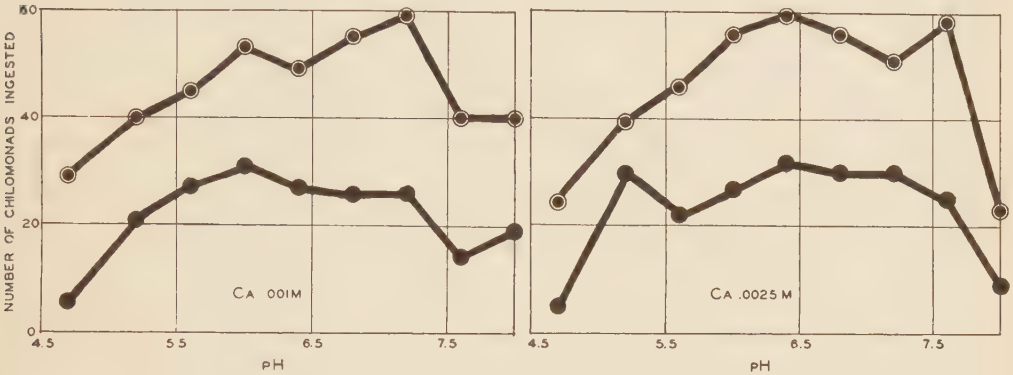

FIG. 4.—The relation between frequency of ingestion of chilomonads by *A. proteus* and hydrogen ion concentration in solutions containing calcium as the only metallic cation. Ordinates, number of chilomonads ingested; abscissae, hydrogen ion concentration in pH units; ●, number ingested in 15 minutes; ◉, 30 minutes. Each point on the curves represents the average for 10 individuals.

Figure 4 also shows that frequency of ingestion of chilomonads was about the same in the weaker and the stronger solutions of calcium, but that the frequency of ingestion at the alkaline limit was much higher in the weaker than in the stronger solution.

It can be seen by comparing Figures 2 and 3 with Figure 4 that the effect of hydrogen ion concentration on frequency of ingestion in calcium solutions differs greatly from that in either sodium or potassium solutions, in that the frequency of ingestion is much higher in the acid and much lower in the alkaline range in the former than in the latter.

The results indicate that the effect of calcium differs qualitatively from either that of sodium or that of potassium. Pitts and Mast (1934) reached the same conclusion in their study on the relations between hydrogen ion concentration in solutions of single salts and rate of locomotion of amoebae.

THE RELATION BETWEEN HYDROGEN ION CONCENTRATION IN SOLUTIONS CONTAINING SODIUM AND CALCIUM IN DIFFERENT PROPORTIONS AND FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus*

The observations described in this section were made with two series of solutions which contained NaH_2PO_4, NaOH, and $CaCl_2$ in water. In both series, 0.005 M NaH_2-

PO_4 and 0.005 M NaOH were mixed in various proportions so as to produce solutions which differed in hydrogen ion concentration as described above. To each solution of one series enough $CaCl_2$ was added to make the concentration of calcium 0.001 M; and to each of the other series, enough to make the concentration of calcium 0.003 M. The calcium and the sodium concentrations of all the solutions were therefore the same in each series. The observations were made as described in the preceding pages. The results obtained are presented in Figure 5.

Figure 5 shows that, as the hydrogen ion concentration decreased, frequency of ingestion increased to a maximum at about pH 6.2, then decreased to a median mini-

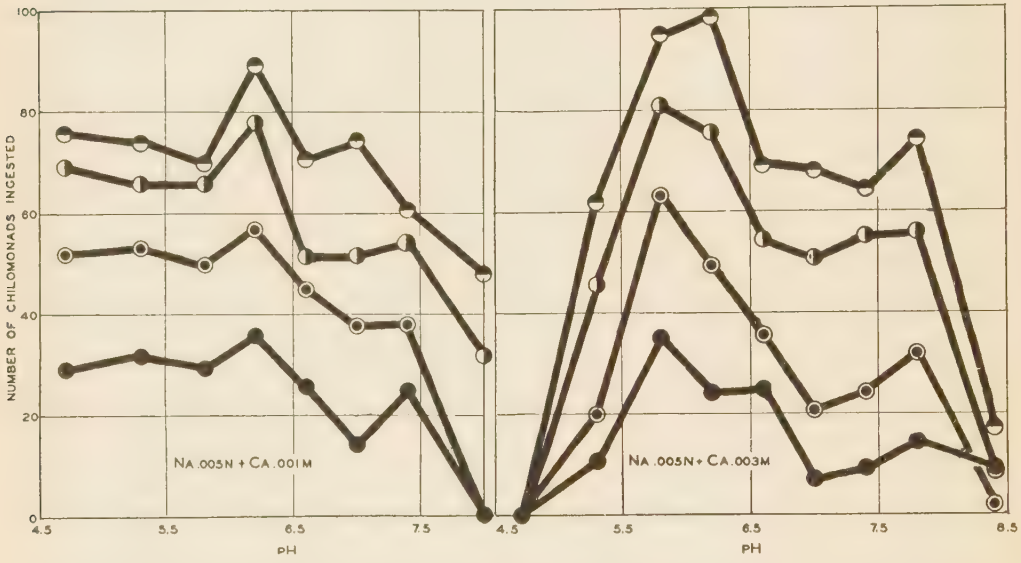

FIG. 5.—The relation between frequency of ingestion of chilomonads by *A. proteus* and hydrogen ion concentration in solutions containing sodium and calcium. *A*, concentration of sodium (0.005 N), calcium (0.001 M); *B*, sodium (0.005 N), calcium (0.003 M); ordinates, numbers of chilomonads ingested; abscissae, hydrogen ion concentration in pH units; ●, number ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals.

mum at approximately pH 7, and then decreased to zero at about pH 8.5. The figure shows that the median minimum is much more marked in the solution containing the higher concentration of calcium than that containing the lower concentration, and that the frequency of ingestion, in the alkaline range, was relatively much higher in the former than in the latter.

By comparing Figure 5 with Figure 2, it will be seen that the addition of calcium 0.001 M to solutions containing sodium 0.005 M had little, if any effect on the frequency of ingestion in the stronger acid solutions (pH 4.7–5.7) but that it caused considerable increase in frequency in the weaker acid and the alkaline solutions (pH 6.2–8.0) with a shift in the maximum from pH 6.0 to 6.2, and that addition of calcium 0.003 M

caused greater increase in frequency in the weaker acid and the alkaline solutions (pH 5.7–8.2) and marked decrease in the stronger acid solutions (pH 4.7–5.5).

The results obtained in this study are similar to those obtained by Pitts and Mast (1934), who found that, as the hydrogen ion concentration increased, in solutions containing 0.005 N sodium and 0.001 M calcium, rate of locomotion increased from zero at pH 4.5 to a maximum at pH 5.9, then decreased to a median minimum at pH 7.0, then increased to a second maximum at pH 7.5 then, decreased again. Hopkins (1928), using modified Ringer solution, and Mast and Prosser (1932), using a solution containing 0.005 N sodium and 0.002 M calcium, obtained similar results. This supports the conclusion reached in the preceding section, that frequency of ingestion of food and rate of locomotion are closely correlated.

TABLE 4

THE EFFECT OF DIFFERENT CONCENTRATIONS OF NaCl ON FREQUENCY OF INGESTION OF CHILOMONADS BY *Amoeba proteus*

Molar Concentration of NaCl	Average Number of Chilomonads Ingested by 10 Amoebae				Numbers Ingested per Individual in 120 Minutes	
	15 Min.	30 Min.	60 Min.	120 Min.	Lowest Number	Highest Number
0.0003	4	26	35	58	35	73
0.0008	37	72	106	185	154	250
0.002	29	55	93	118	81	163
0.005	29	57	90	110	56	225
0.008	29	45	90	100	62	165
0.01	19	43	86	86	65	120
0.02	25	38	44	55	40	82
0.03	4	7	8	10	0	30

THE RELATION BETWEEN NaCl, KCl, $MgCl_2$, AND $CaCl_2$ IN DIFFERENT CONCENTRATIONS AND THE FREQUENCY OF INGESTION OF FOOD BY *Amoeba proteus* and *A. dubia*

The experiments described in this section were made with different concentrations of the chlorides of sodium, potassium, magnesium, and calcium. The observations were made as described in the preceding pages. The results are presented in Table 4 and Figures 6 and 7.

Figure 6 and Table 4 show that, as the concentration of NaCl increased from 0.0003 M, frequency of ingestion of chilomonads very rapidly increased to a maximum at 0.0008 M, then very gradually decreased nearly to zero at 0.03 M, and that at the optimum concentration an average of 185 chilomonads were ingested by each amoeba in 120 minutes.

Figure 6 also shows that the relation between frequency of ingestion and concentration in solutions of KCl is similar to what it is in solutions of NaCl but that the range of concentrations over which ingestion occurs is very much shorter in the former than it is in the latter, and that frequency of ingestion is consistently lower throughout the range, the average maximum frequency being 185 in 120 minutes in NaCl and only 90

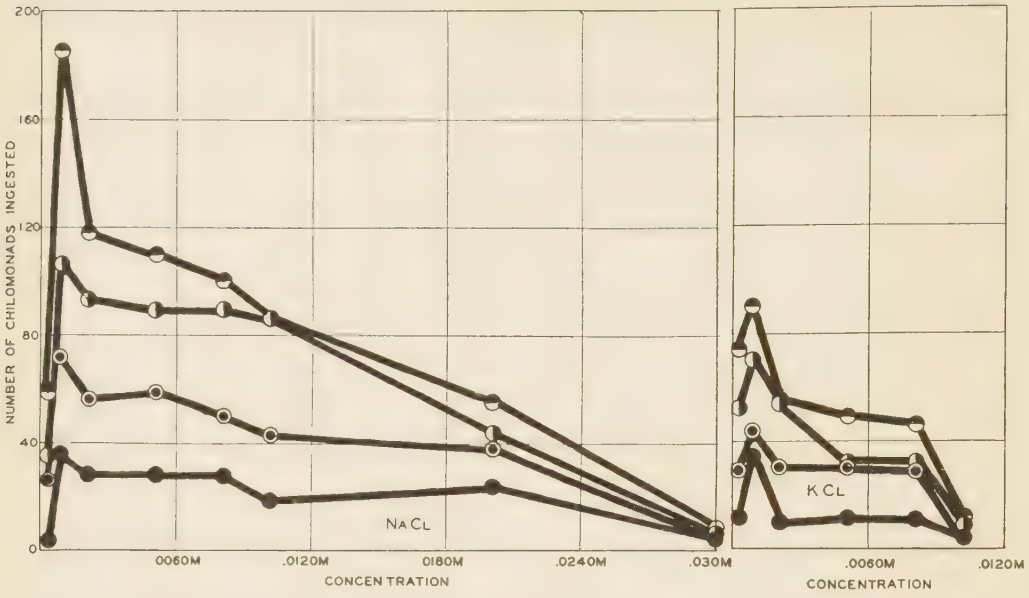

FIG. 6.—The effect of concentrations of salts on frequency of ingestion of chilomonads by *A. proteus*. *A*, NaCl; *B*, KCl; ordinates, number of chilomonads ingested; abscissae, molar concentration; ●, number ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals.

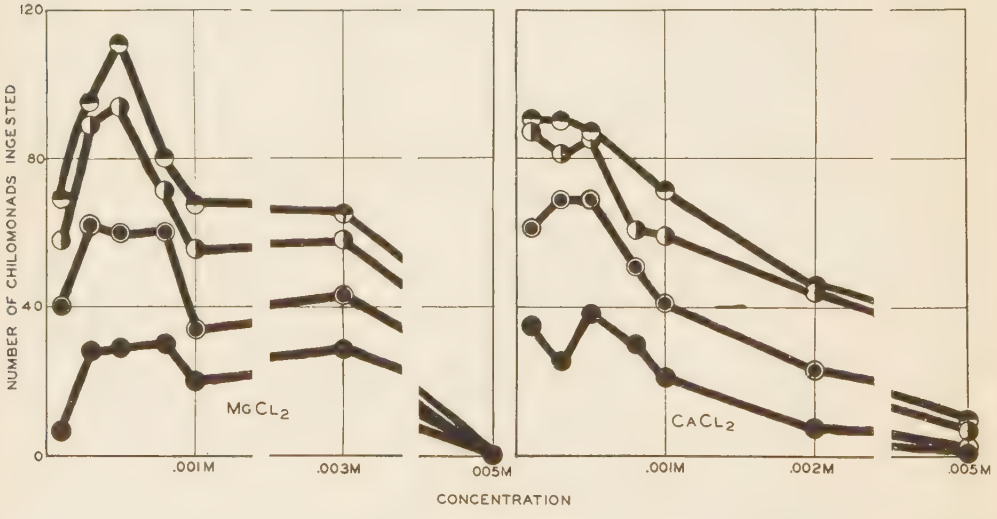

FIG. 7.—The effect of different concentrations of salts on frequency of ingestion of chilomonads by *A. proteus*. *A*, $MgCl_2$; *B*, $CaCl_2$; ordinates, number of chilomonads ingested; abscissae, molar concentration; ●, number ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes.

in KCl. The results consequently show that potassium solutions are much less favorable for ingestion than sodium solutions.

These results agree with those obtained by Mast and Prosser (1932) in their study of amoeboid locomotion in different concentrations of KCl. They found that it is extremely difficult to obtain active amoebae in any concentration of KCl, except 0.001 N, and that in this concentration locomotion continued only a short time. Heilbrunn and Daugherty (1932) contend that potassium has strong liquefying action on the plasmagel of amoebae and that it is consequently highly toxic.

Figure 7 shows that, as the concentration of $MgCl_2$ increased from 0.0001 M, frequency of ingestion increased to a maximum at 0.0005 M and then decreased to zero at 0.005 M. It also shows that the relation between frequency of ingestion and concentration in solutions of $CaCl_2$ is similar to what it is in solutions of $MgCl_2$, but that in the lowest concentration used, it is much higher in $CaCl_2$ than in $MgCl_2$ and at optimum concentration somewhat higher in $MgCl_2$ than in $CaCl_2$.

The results presented in Figures 6 and 7 show that the optimum concentration for ingestion of chilomonads by amoebae is at 0.0008 M for NaCl and KCl, at 0.0005 for $MgCl_2$, and probably somewhat lower for $CaCl_2$ The results show also that the maximum frequency of ingestion is highest in NaCl solutions (185), lower in $MgCl_2$ (111), still lower in $CaCl_2$ (91), and lowest in KCl (90); that the maximum concentration in which ingestion occurs is highest in NaCl (0.03 M), much lower in KCl (0.01 M), and still lower in $MgCl_2$ and $CaCl_2$ (0.005 M); and that the minimum concentration in which ingestion occurs is much lower in $MgCl_2$ and $CaCl_2$ (0.0001 M), than in NaCl and KCl (0.0003 M). They show, moreover, that the range of concentration over which ingestion occurs is much greater in NaCl (0.03 M) than in KCl (0.01 M), and much greater in KCl than in $MgCl_2$ and $CaCl_2$ (0.005 M), i.e., that it is more than six times as great in NaCl as in $MgCl_2$ or $CaCl_2$, and about three times as great as in KCl.

The facts that the frequency of ingestion at optimum concentration is approximately twice as high in NaCl as it is in KCl, $MgCl_2$ or $CaCl_2$, and that the range of concentrations over which ingestion occurs is approximately six times as great in NaCl as it is in $MgCl_2$ or $CaCl_2$ and approximately three times as great as it is in KCl, show that ingestion is not specifically correlated with any of the properties common to all these salts—as, for example, osmosis.

THE RELATION BETWEEN OSMOTIC CONCENTRATION AND FREQUENCY OF INGESTION OF CHILOMONADS BY *Amoeba proteus* AND *A. dubia*

Observations on frequency of ingestion by *A. proteus* and *A. dubia* were made in lactose solution, Chalkley solution (see p. 4), Hahnert solution, and Hahnert solution containing various quantities of lactose. The results are presented in Table 5 and Figure 8.

Table 5 shows that in *A. dubia* the frequency of ingestion was zero in lactose solutions of all concentrations tested, that it was much higher in Chalkley solution than in Hahnert solution, that addition of lactose to Hahnert solution caused marked increase in frequency of ingestion, and that, as the concentration of lactose increases, the frequency increases to a maximum and then decreases.

Figure 8 shows that the results obtained with *A. proteus* are essentially the same as those obtained with *A. dubia*, but that the frequency of ingestion was very much lower, the maximum in 2 hours being more than 400 for the former and only 99 for the latter.

TABLE 5

THE RELATION BETWEEN FREQUENCY OF INGESTION OF CHILOMONADS BY *Amoeba dubia* AND OSMOTIC PRESSURE

Molar Concentration of Solution Used	Average Number of Chilomonads Ingested by 10 Amoebae				Numbers per Individual Ingested in 120 Minutes	
	15 Min.	30 Min.	60 Min.	120 Min.	Lowest Number	Highest Number
0.0001 M lactose	0	0	0	0	.....	.....
0.0005 M lactose	0	0	0	0	.....	.....
0.001 M lactose	0	0	0	0	.....	.....
0.002 M lactose	0	0	0	0	.....	.....
0.003 M lactose	0	0	0	0	.....	.....
0.035 M lactose	0	0	0	0	.....	.....
Chalkley solution	71	137	196	374	358	396
Hahnert solution	34	72	135	193	172	215
Hahnert solution plus 0.03 M lactose	54	123	171	263	240	325
Hahnert solution plus 0.05 M lactose	60	150	232	336	275	405
Hahnert solution plus 0.07 M lactose	51	68	153	169	100	270

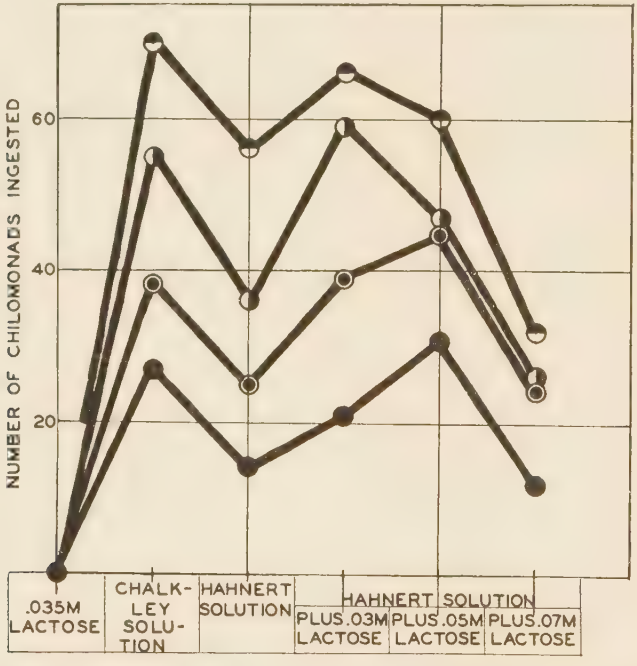

FIG. 8.—The relation between frequency of ingestion of chilomonads by *A. proteus* and osmotic pressure. Ordinates, number of chilomonads ingested; abscissae, molar concentration of solutions; ●, number of chilomonads ingested in 15 minutes; ◉, 30 minutes; ◑, 60 minutes; ◓, 120 minutes. Each point on the curves represents the average for 10 individuals.

The fact that the frequency of ingestion was higher in Hahnert solution plus lactose than in Hahnert solution indicates that ingestion is correlated with osmotic concentration. The fact that it was zero in lactose solutions and higher in Chalkley solution than in Hahnert solution plus lactose (0.03 M), which was osmotically equal to Chalkley solution, indicates that the effect of salts on ingestion is not entirely due to their osmotic action. The marked difference in the frequency of ingestion in Chalkley and Hahnert solutions is doubtless due to the fact that the former contains NaCl and the latter does not.

DISCUSSION

Mast made a thorough study of the effect of salts, nonelectrolytes, and hydrogen ion concentration on attachment of *A. proteus* to the substratum. He maintains that nonelectrolytes have no effect on attachment, but that all salts greatly facilitate it and that this is not closely correlated with their concentration or the hydrogen ion concentration of the solutions. He says (1929, p. 375):

> All the salts tested (17 in number) greatly decrease the time required for attachment and increase the firmness of attachment. All have the same effect but not in the same concentration
>
> The effect of NaCl and KCl increases very gradually from N/20 to a maximum at about N/100, after which it decreases very gradually until about N/1000 is reached and then very rapidly. The effect of $CaCl_2$ increases rapidly from N/20 to about N/60 then slowly to a maximum at about N/200; after which it decreases very slowly until about N/40,000 is reached and then very rapidly. There is very little difference in the effect on attachment of NaCl and KCl in concentrations ranging from N/40 to N/800 and of $CaCl_2$ in concentrations ranging from N/80 to N/40,000. The effect of $CaCl_2$ is considerably less than that of NaCl and KCl in the stronger and much greater in the weaker solutions tested. All three salts, however, still have a definite effect in the weakest solution tested, N/100,000.
>
> The optimum hydrogen ion concentration for attachment in balanced solution appears to be between pH 6.6 and 7; but there is remarkably little difference over a very wide range, i.e. from pH 4.6 to 8.2.

He found no indication whatever of a median minimum at neutrality.

By comparing these results with those presented in the preceding pages, it is at once evident that, while the effect of nonelectrolytes on attachment seems to be the same as it is on ingestion, the effects of salts and hydrogen ions on ingestion differ greatly from their effects on attachment, and that this is particularly striking in reference to a hydrogen ion concentration of pH 7, in which ingestion was found to be maximum and attachment minimum.

This strongly indicates that, while ingestion is doubtless dependent upon attachment, in accord with the views of Mast and Hahnert (1935), these two phenomena are not closely correlated.

We have demonstrated that the relations between temperature, properties and concentration of salts, and hydrogen ion concentration and frequency of ingestion of food by *Amoeba* are essentially the same as the relation between these environmental factors and the rate of locomotion. This indicates that the factors which control ingestion are fundamentally the same as those which control locomotion.

Pitts and Mast (1934) considered in some detail the mode of action of environmental factors in controlling locomotion. They accounted for some phases of the phenomena involved; but they could give no consistent explanation of the relation between hydro-

gen ion concentration and rate of locomotion in balanced salt solution, especially the extraordinary reduction in rate at neutrality.

We have no suggestions to offer concerning the mode of action of environmental factors in controlling ingestion except that it probably is essentially the same as it is in reference to locomotion, and we have nothing of value to add to the discussion of Pitts and Mast concerning this.

The results presented demonstrate clearly that in optimum concentration sodium is much more favorable for ingestion than potassium, calcium, or magnesium. We have no suggestions concerning the causes of this.

SUMMARY

1. A quantitative method was devised and used in studying the frequency of ingestion of food by amoebae.

2. Frequency of ingestion varies inversely with the amount of food in the amoebae, but there can be large variations in the abundance of food in the surrounding medium without appreciably affecting frequency of ingestion.

3. As the temperature increases, frequency of ingestion increases from zero at 0° C to a maximum at 25°, and then decreases to zero at about 40°. There is some evidence of a median minimum at 30°.

4. As the hydrogen ion concentration decreases in solutions in which sodium is the only metallic cation, frequency of ingestion increases from zero at pH 4.4 to a maximum at approximately pH 6.0, then decreases to zero at pH 7.6 to 8.3. There is strong indication of a median minimum between pH 6.5 and 7.0. The results indicate that in solutions in which the concentration of sodium is 0.005 N, frequency of ingestion is closely correlated with the hydrogen ion concentration, but not in solutions in which the concentration of sodium is 0.002 N.

5. The relation between hydrogen ion concentration and frequency of ingestion in solutions in which potassium is the only metallic cation is similar to that in solutions in which sodium is the only metallic cation, but the range over which ingestion occurs is much shorter and the frequency of ingestion is much lower.

6. In solutions in which calcium is the only metallic cation, frequency of ingestion increases from about 0.9 chilomonads per minute at pH 4.7 to a maximum of 1.8 chilomonads per minute at approximately pH 6.5, then decreases very slowly until pH 8.0 is reached, and then drops rapidly to zero.

7. In solutions in which sodium and calcium are the only metallic cations, frequency of ingestion increases very rapidly from zero at pH 4.5, to a maximum at pH 6.2 then decreases to a marked median minimum at pH 7.0, then increases to a secondary maximum approximately at pH 7.7, then decreases nearly to zero at pH 8.4. The median minimum and the secondary maximum in the alkaline region of the range are much more pronounced in solutions containing Na 0.005 N and Ca 0.003 M than in solutions containing Na 0.005 N and Ca 0.001 M.

8. In solutions of NaCl, as the concentration increased, frequency of ingestion increased very rapidly from about 0.49 chilomonads per minute at 0.0003 M, to a maximum of 1.54 chilomonads per minute at 0.0008 M, and then decreased slowly to zero at 0.03 M.

9. The relation between concentration and frequency of ingestion in solutions of KCl is essentially the same as in solutions of NaCl; but the frequency of ingestion is much

lower, and the range over which ingestion occurs is only about one-third as long, the maximum concentration being 0.03 M in the one and only 0.01 M in the other.

10. In solutions of $MgCl_2$, as the concentration increases, the frequency of ingestion increases rapidly from 0.59 chilomonads per minute at 0.0001 M, to a maximum of 0.83 chilomonads at 0.0005 M, and then decreases gradually to zero at 0.005 M.

11. The relation between concentration and frequency of ingestion in solutions of $CaCl_2$ is essentially the same as in solutions of $MgCl_2$ except that frequency in the lowest concentration tested is considerably higher.

12. The range of concentration over which ingestion occurs in solutions of $MgCl_2$ or $CaCl_2$ is only about one-sixth as long as that over which it occurs in solutions of NaCl. The cause of this difference is not clear.

13. The relation between temperature, salts, and hydrogen ion concentration and the frequency of ingestion of food is essentially the same as the relation between these environmental factors and rate of locomotion. Frequency of ingestion and rate of locomotion are therefore closely correlated.

14. Frequency of ingestion of chilomonads by *A. proteus* and *A. dubia* is zero in solutions of lactose in all concentrations, but is it higher in Hahnert solution plus lactose than in Hahnert solution without lactose. This indicates that osmosis is involved in feeding.

15. The frequency of ingestion is much higher in Chalkley solution than in Hahnert solution plus lactose 0.003 M (osmotically equal to Chalkley solution). This is doubtless due to the fact that the former contains NaCl and the latter does not.

16. The frequency of ingestion is much higher in *A. dubia* than in *A. proteus*. The maximum number ingested by an individual of the former was 100 in 15 minutes, 195 in 30 minutes, 366 in 60 minutes, and 405 in 120 minutes; and by one of the latter, only 48 in 15 minutes, 95 in 30 minutes, 130 in 60 minutes, and 250 in 120 minutes.

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VITA

Richard A. Fennell was born in Decatur, Alabama, on April 19, 1903. His elementary and secondary education was received in the Grammar Schools, and the Central High School in Decatur. In the fall of 1923 he entered Birmingham-Southern College, and received the A.B. degree in 1927. He was retained as laboratory instructor during 1927–28. He taught Biology in Woman's College of Alabama during the summer of 1928.

In the fall of 1928, he entered Duke University as a graduate student in Zoölogy, and remained until the spring of 1930. During the summer of 1930 he took a course in Invertebrate Zoölogy at the Marine Biological Laboratory. The following fall he returned to Birmingham-Southern College as Instructor in Biology, and remained there until the spring of 1933.

He entered the Johns Hopkins University as a graduate student in Zoölogy in the fall of 1933. For the past three years he has been an assistant in General Biology. During the summers of these years he carried on research at the Johns Hopkins University and the Marine Biological Laboratory.